IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	Confirmation No.: 8688
DEVIDAL et al.	Group Art Unit: 1616
Application Serial No.: 10/500,868	Examiner: Alstrum Acevedo, J.H.
Filed: July 21, 2004	Attorney Docket No.: 021305-00201

For: METHOD FOR TREATMENT OF A REGION OF THE SKIN OF A HUMAN SUBJECT, IN PARTICULAR COSMETIC TREATMENT BY THE TRANS-EPIDERMAL ROUTE USING PROJECTION OF A LIQUID UNDER PRESSURE

DECLARATION UNDER 37 C.F.R. § 1.132

I, ALAIN CORRE, hereby declare that:

1. I am one of the inventors of the invention entitled "METHOD FOR TREATMENT OF A REGION OF THE SKIN OF A HUMAN SUBJECT, IN PARTICULAR COSMETIC TREATMENT BY THE TRANS-EPIDERMAL ROUTE USING PROJECTION OF A LIQUID UNDER PRESSURE," as claimed in U.S. Patent Application Serial No. 10/500,868, filed on July 21, 2004.

2. This Declaration discusses the enclosed four references, which I believe support the idea that it is essential to have a pressure higher than 10 bars to obtain a penetration of active substances in the papillary dermis. The four references are as follows:

- D1: Divaris, M., "Dermal improvement via aquapressure: DIVA Protocol," International Journal of Cosmetic Surgery and Aesthetic Dermatology, August 2003.
- D2: Falson, F. et al., "Ex-vivo study of the cutaneous penetration of sodium ascorbyl phosphate contained in two cosmetic formulations administered by Acqua-Peel®"
- D3: Pirot F. et al., "Hydrodynamic sodium ascorbyl phosphate delivery into the skin"
- D4: Falson, F. et al., "Ex Vivo Study of the Functional Condition of the Skin Following Administration of Demineralised Water"

3. I submit that Divaris, M., "Dermal improvement via aquapressure: DIVA Protocol," International Journal of Cosmetic Surgery and Aesthetic Dermatology, August 2003 (hereinafter "D1") discloses a method involving the step of abrading the stratum corneum and spraying a pressurized liquid to the skin. I submit that D1 shows that administering a liquid at a pressure of between 15 to 18 bars results in transcutaneous absorption, without damaging the skin (see page 186) and that skin perforation occurs at about 20 bars.

4. I submit that Falson, F. et al., "Ex-vivo study of the cutaneous penetration of sodium ascorbyl phosphate contained in two cosmetic formulations administered by Acqua-Peel®" (hereinafter "D2") discloses a method involving abrasion of the epidermis of pig ear skin and administration of a liquid composition by means of

microjets. I submit that Figures 8 and 9 of D2 show that there is a correlation between the cutaneous concentration of the liquid composition in abraded skin after administration and the pressure at which the liquid composition is administered. I submit that Figure 8 shows that the increase in the concentration of the liquid composition in the abraded skin (µg/g) was much greater when administered at a pressure of from 10 to 20 bar, compared to that administered at a pressure from 0 to 10 bar.

5. I submit that Pirot F. et al., "Hydrodynamic sodium ascorbyl phosphate delivery into the skin" (hereinafter "D3"), and in particular, Figure 1, shows that the penetration of the liquid composition into abraded skin is a function of the micro-jet pressure at which the composition is administered. I submit that D3 shows that the content of composition in the skin increases with an increase in pressure.

6. I submit that Falson, F. et al., "Ex Vivo Study of the Functional Condition of the Skin Following Administration of Demineralised Water" (hereinafter "D4"), which shows that the administration of demineralised water at a low pressure does not appear to modify the functional condition of the skin. In contrast, I submit that D4 shows that administration of a liquid composition at higher pressures (for example, above 5 bars) appears to modify the functional properties of the skin and, in particular, the skin barrier function.

7. I believe that it is instrumental to have a pressure between 10 to 20 bars to get a penetration of active substance in the papillary dermis. I believe that this has been proven, because under 10 bars, there is no significant penetration and above 25 bars, there is a strong risk of cutting the skin.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent(s) issuing therefrom.

Date: Sept 1st 2008

Alain Corre

INTERNATIONAL JOURNAL OF COSMETIC SURGERY and AESTHETIC DERMATOLOGY
Volume 5, Number 2, 2003

Dermal Improvement via Aquapressure: DIVA Protocol

MARC DIVARIS, M.D.

ABSTRACT

A new and innovative combined technique is now available to improve the skin texture and rejuvenate the skin. The principle involves a very superficial abrasion of the stratum corneum with fruit pit powder followed by projection of a very powerful microjet of water containing active principles including antioxidants, moisturizing agents, and other ingredients. Biopsies and clinical studies show improvement in the skin, proving that it is not necessary to create a dermal wound to obtain dermal modification.

INTRODUCTION

THE KNOWN ALTERNATIVE METHODS (laser, peeling, etc.) used to resurface the skin allow regeneration of the dermal and epidermal components by means of cicatrization from remaining cells.

The biological repair process varies according to the depth level reached. The purpose of the dermal improvement via aquapressure (DIVA) process is not to obtain cicatrization but rejuvenation of the tissues. After simple elimination of the corneous and superficial layers, mechanical stimulation by hyperpressure with antioxidant-loaded water will lead to dermal modifications. This non-invasive technique shows that it is not necessary to create a dermal wound to obtain dermal modification.[1]

CONCEPTS

DIVA is a method that associates two techniques that have in common stimulation of the dermis: by molecular and mechanical action. The material used is a device that combines microabrasion and liquid hyperpressure. This new patented device called Water-Beam (Medicamat S.A., France) works with two different hand-pieces: one with abrasive powder and one with a high-pressure water brush.

The first technique used is microepidermabrasion (Fig. 1). This soft abrasion, practically painless and achieved without local anesthesia, involves projecting biological abrasive powder made of fruit pits, instead of aluminium hydroxide microcrystals. The fruit pit powder used has a particle size between 110 and 140 μm and is instantly sucked in a waste canister. A superficial abrasion of the stratum corneum, and often of the stratum granulosum, will be achieved; the basal and spinosum layers are not touched.

The second step involves water hyperpressure: projection over the skin, and with high pressure, of plain water or physiological saline solution. Known for years in industry for cutting plastics or metals, the water jet cutting method has had surgical applications on soft tissues such as the liver. In this new applica-

Associate Clinical Professor of Maxillo-Facial and Plastic Surgery, Salpêtrière Hospital, Paris, France.

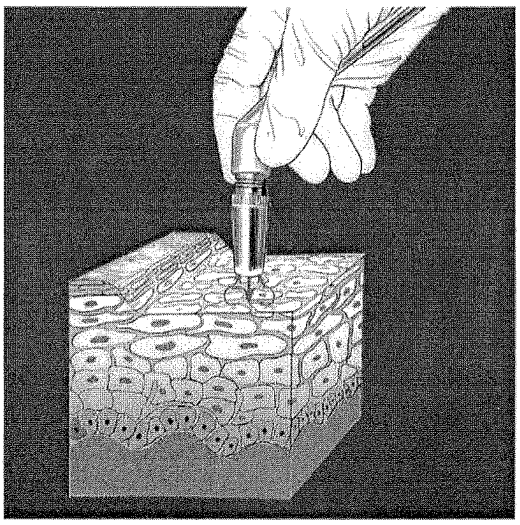

FIG. 1. DIVA protocol of microepidermabrasion is achieved with projecting biological abrasive powders; the basal and spinosum layers are not touched.

tion, the power used is greater because the skin is very elastic. A hand piece with different nozzles brings the products under pressure; this water is immediately captured by the adjacent vacuum system.

The power of this method is calculated to be at the limit of the cut or frank abrasion without, however, any risk of skin perforation. The power used is between 15 and 18 bars: skin perforation occurs at approximately 20 bars. The vacuum has the sole purpose of capturing the water and skin scraps. The microjet used is a 5 mm water paint brush passed over the zones to be treated. Used alone, the efficacy of the water pressure is limited. We obtained excellent clearing of the skin and suppression of blackheads (Figs. 2, 3).

More interesting is that the stratum corneum is unharmed to the high water pressure; the skin was not damaged by the aquabrasion, which proves the adequate cohesion of the corneocytes.

Water projection over a fragile epidermis after microepidermabrasion results in transcutaneous absorption. This is desirable because the water, as a moleculular vehicle, allows the active agents to penetrate the epidermis (Fig 4). The results of four different DIVA protocols each different, are reviewed below.

RESULTS

Lidocaine

This test demonstrates the molecular penetration of lidocaine by hyperpressure. First, the classic epidermabrasion eliminates the stratum corneum. Then, I performed an epicritical cutaneous test with a needle, revealing a stronger sensitivity at the center of the abrasion than at the sides of the area. This indicates an irritation of the nerve-free intraepidermal termination (Fig. 5). Next, three doses of lidocaine are injected in the physiological serum reservoir and the microjet is propelled over the previously abraded surface. The result obtained is hypoesthesia of the treated surface, demonstrating that the lidocaine molecules did penetrate intraepidermally. If the needle is inserted more deeply, the patient feels a little pain. This indicates that the molecular penetration did not traverse the dermoepidermal membrane.

FIG. 2. Before treatment: cohesion of the stratum corneum and blackhead composed of lipidic substances and keratinocyte scraps.

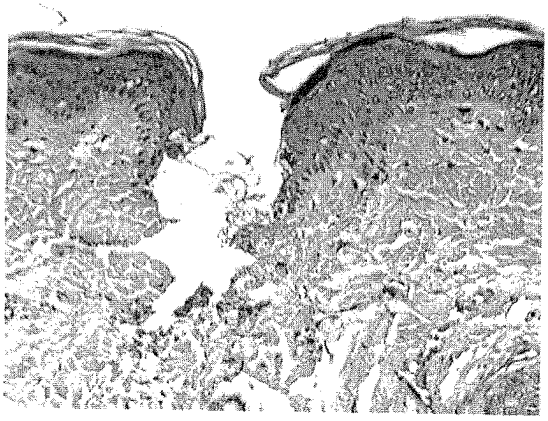

FIG. 3. After water pressure: the force is such that it allows extraction of the blackhead and elimination of dead cells. The stratum corneum is unharmed by the high water pressure.

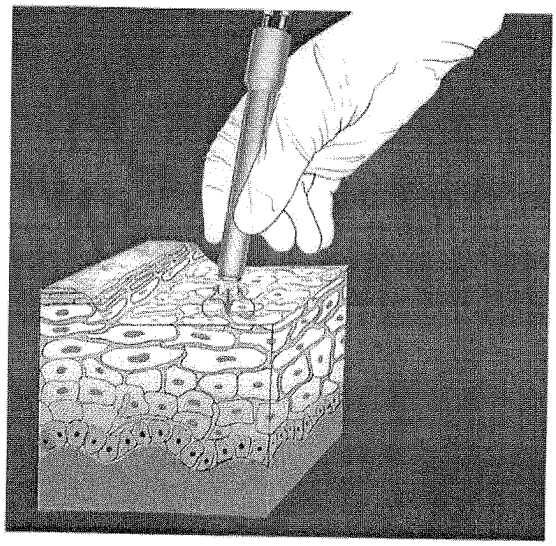

FIG. 4. Water projection after microepidermabrasion results in transcutaneous absorption.

Cutaneous penetration

To demonstrate the depth reached by the molecules, I performed a clinical test using china ink with carbon as a indicator. The histological results are revealing. One can note the ablation of stratum corneum as well as the superficial part of the epiderm. The voluminous carbon molecules penetrated the basal epidermis as if they had gone through the interkeratinocyte desmosomes. The dermoepidermal membrane was untouched, as if it had been an obstacle.

There is also no trace of keratinocytes inside the dermis. The carbon molecules penetrated zones of lesser resistance without damaging this basal epithelium. It is interesting to note that if we abrade the epidermis more completely, the carbon molecules can even penetrate the superficial dermis after microjet treatment (Fig. 6).

This experience is the irrefutable proof of

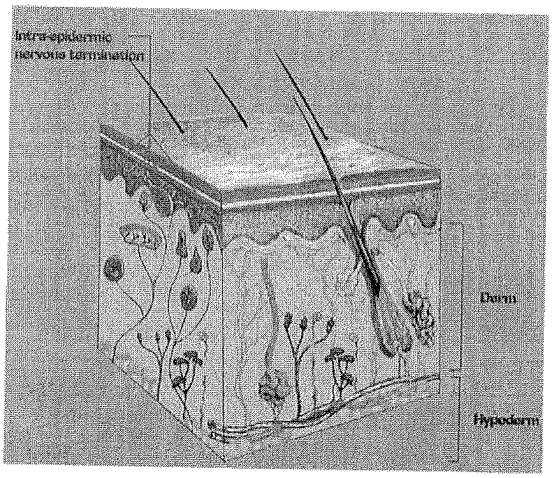

FIG. 5. Note the nerve-free intraepidermal terminations.

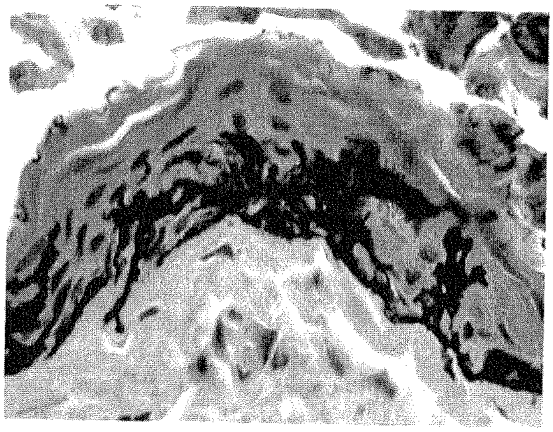

FIG. 6. Carbon molecules penetrate by hyperpressure through zones of lesser resistance without damaging the basal epithelium.

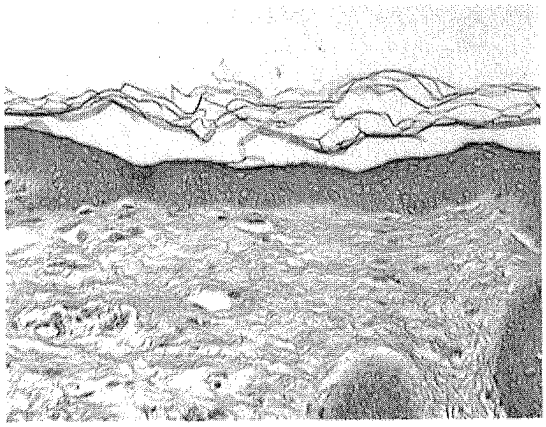

FIG. 7. Skin biopsy in a male patient before treatment.

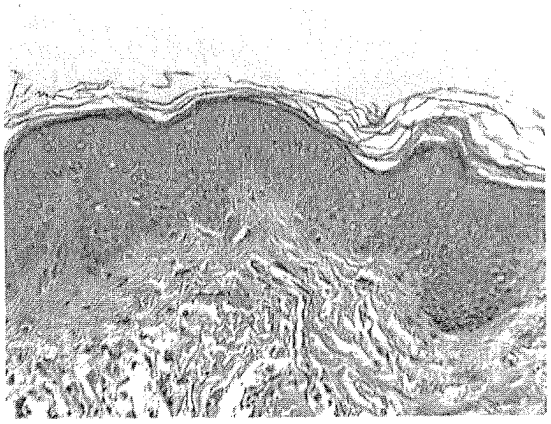

FIG. 8. Biopsy in a male patient after five DIVA passages.

penetration of active agents by water pressure after the stratum corneum has been abraded.

Histological protocol

To appreciate the histological modifications after treatment with DIVA, I performed two biopsies on two patients before treatment and after five treatments. The biopsy results show not only a normalization of the stratum corneum and its network, but also a thickening of the epidermis[2] and the reappearance of the papillary dermal crests (Figs. 7–10).

Chemical protocol

The goal of a chemical protocol performed on two types of skin was to compare the efficiency of microjet after abrasion in sample skins. Different histological and biochemical parameters were studied, using an experimental sample of skin that was kept alive.

Histological evaluation of glycosaminoglycans was achieved through Hales's dye. A semiquantitative evaluation using scores provides evidence for eventual modifications in the quantity of glycosaminoglycans in the dermis. We observed that the intensity scores of

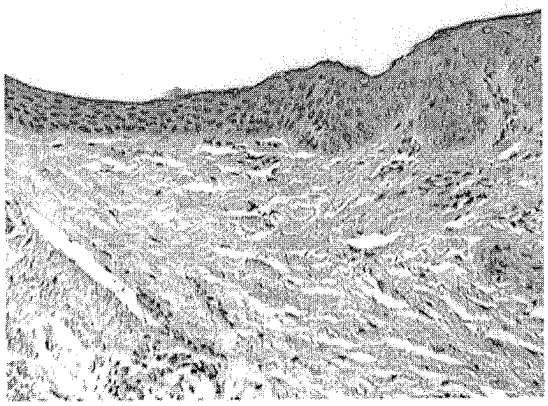

FIG. 9. Biopsy in a female patient before treatment.

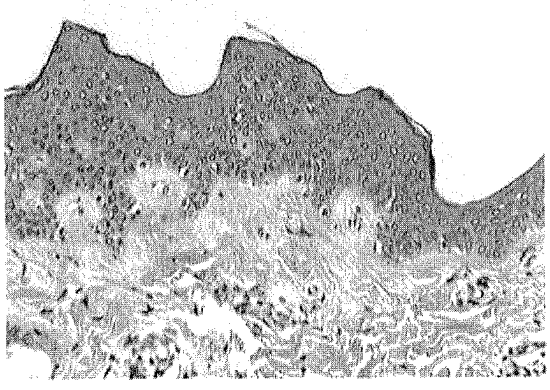

FIG. 10. Biopsy in a female patient after five DIVA passages.

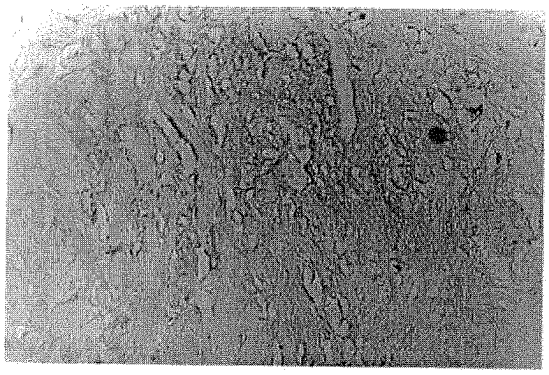

FIG. 11. Before treatment, blue coloration indicates presence of glycosaminoglycans.

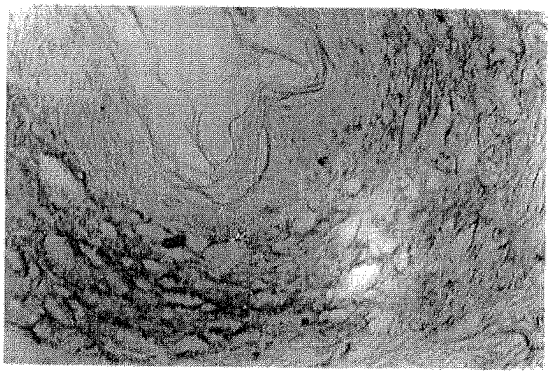

FIG. 12. After treatment, a bright blue coloration indicates a glycosaminoglycans synthesis potentialized by the microjet.

the dye in the presence of glycosaminoglycans are greater for abraded skin with the microjet. One observes in this case a bright blue coloration, which indicates the presence of glycosaminoglycans, the sponges of the dermis that ensure its hydration (Figs. 11, 12).

The dosage of collagen synthesis was evaluated with tritium proline added to the culture fluid. Our aim was to evaluate the antiaging efficacy of a formula containing 0.05% retinaldehyde and pretocopheryl (Ystheal). The results show an increase in the incorporation of tritium proline at the dermal fibroblasts treated with Ystheal after abrasion, in comparison with both sample skin and untreated abraded skin. The microjet seems to have potentialized the effect of the abrasion, since this combination showed the highest collagen synthesis (Table 1).

DISCUSSION

DIVA combines two complementary and interactive technologies. The first—microabrasion—has been known for some time and is experiencing a renewal of interest because of its low morbidity and good results. The other, however, is new. This new method of transcutaneous penetration opens many possibilities as far as the molecules to be used. During the different protocols examined, I first eliminated any chemical agent to avoid a disguised peeling. My choice tended towards antiradical substances. The aggression of the free radicals is a major factor in skin aging. The skin includes a conjunctive dermis with low endogenous antiradical defenses and enzymatic systems.

Applying topically substances with antiradical properties is useful to compensate the lack of these substances and also to act directly on skin aging. We know aging is induced by ultraviolet solar radiation. It can also result from the progressive and ineluctable natural degradation of cutaneous structures. The antiradicals used included a mixture of vitamins A and C, along with oligoelements (zinc and silicium). Determining the ingredients and the balance between normally recommended doses is difficult. There might also exist eventual interactions between various substances. Yet studies in humans are sparse, and their protocols vary greatly, which makes the results difficult to compare. A study is presently in process involving daily application of a vitamin and oligoelement-based cream between each DIVA

TABLE 1. DOSAGE OF COLLAGEN SYNTHESIS BY WEBSTER TECHNIQUE

–Abrasion + microjet + Ystheal® cream: 2168 cpm/mg
–Abrasion + Ystheal® cream: 1640 cpm/mg
–Abrasion only: 1157 cpm/mg
–No abrasion witness skin: 1242 cpm/mg

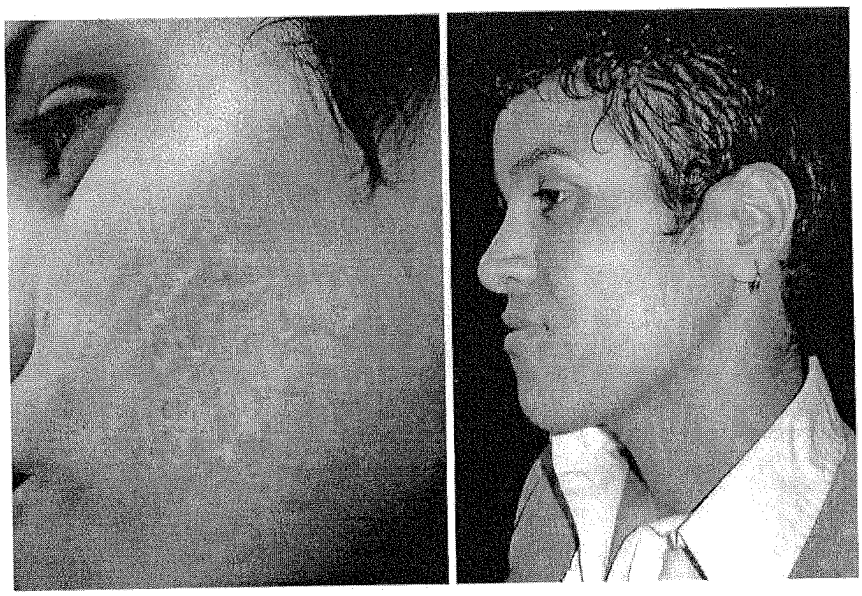

FIG. 13. A 28-year-old woman with acne scars before treatment (**left**) and after 5 weekly DIVA treatments (**right**).

treatment. A modification of the dermis by activation of the fibroblasts, augmentation of collagen synthesis, and augmentation of dermal hydration can be observed.

CONCLUSION

DIVA modifies the dermis by its epidermal action. The Water-Beam biological technique using water, fruit, vitamins, and oligoelements has various advantages: (Figs 13, 14).

- superficial wrinkle treatment
- improvement of the quality of the skin
- improvement of skin moisture
- penetration of molecules with potentialization of their action

Another advantage is the possibility of use by

FIG. 14. A 55-year-old woman before treatment (**left**) and after 4 weekly DIVA treatments (**right**).

each practician according to his or her experience and preferences with antioxidants (or others) that will act directly inside the epidermis homogeneously. This treatment can in no way replace other resurfacing techniques such as laser for the treatment of deep wrinkles, for example.

REFERENCES

1. Rubin, M.G. and Greenbaum, S.S.: Histologic effects of aluminium oxide microdermabrasion on facial skin. J Aesth Dermatol Cosmet Surg. 2000;1(4):237–240.
2. Freedman, E., Rueda-Pedraza, S.P., and Waddell: The epidermal and dermal changes associated with microdermabrasion. Dermatol Surg 1998;27(12):1031–1034.

Address reprint requests to:
Marc Divaris, M.D.
59 Avenue Augustin Dumont
92240 Malakoff, France

E-mail: medicam@club-internet.fr

CLAUDE BERNARD UNIVERSITY - LYON 1

Faculty of Pharmacy

Pharmaceutical and Biological Sciences Institute

8, Avenue Rockefeller - 69373 LYON Cedex 08

Tel. +33 4 78 77 71 67 - Fax +33 4 78 77 72 47

PHARMACOTECHNOLOGY, BIOPHARMACY AND COSMETOLOGY LABORATORY

F. FALSON, University Professor
F. PIROT, Senior Lecturer, Practitioner attached to the Lyon Civil Hospices

REPORT: EX VIVO STUDY OF THE CUTANEOUS PENETRATION OF SODIUM ASCORBYL PHOSPHATE CONTAINED IN TWO COSMETIC FORMULATIONS ADMINISTERED BY ACQUA-PEEL®

Biological samples

The biological samples necessary for carrying out the ex vivo tests come from a sample of pig ears carried out in the Physiology Laboratory of Dr Q. TIMOUR (CLAUDE BERNARD UNIVERSITY - LYON 1). The recovery of the pig ears was carried out by the main investigator with the agreement of the manager of the Physiology Laboratory, 16 hours before the test. The ears were stored at +4°C until the start of the test. The ears not used in the study have been stored at -18°C.

Products studied:

- Sodium ascorbyl phosphate (SAP),
- Anti-ageing product containing 3% sodium ascorbyl phosphate (SAP)(MBAAA009),
- Bleaching product containing 3% sodium ascorbyl phosphate (SAP) (MBE013)

Galenic form:

- Sodium ascorbyl phosphate: white powder
- Anti-ageing product (MBAA009): pink liquid
- Bleaching product - (MBE013): yellow liquid

Packaging:

- Sodium ascorbyl phosphate: 5 g in a plastic sachet
- Anti-ageing product (MBAA009): 500 ml bottles,
- Bleaching product (MBE013): 500 ml bottles

Storage:

- Away from light and heat (4°C)

It was the responsibility of the Study Supervisor to determine the identity, the physical and chemical characteristics and all the criteria that made it possible to identify the products studied.

TECHNICAL PROTOCOL

1. ABRASION OF TEH EPIDERMIS OF THE PIG EAR SKIN BY BIOPEEL®

The purpose of the abrasion of the epidermis of the pig ear skin was (i) to reproduce ex vivo the abrasion of the epidermis carried out in vivo in humans, (ii) to facilitate the cutaneous penetration of active products in the dermis.

The abrasion of the epidermis of the pig ear skin was carried out ex vivo on samples of skin, with a surface area of approximately 2.54 cm^2, using apricot kernel powder delivered by Biopeel®. The abrasion was carried out in accordance with the following procedure:

- 5 horizontal movements from left to right,
- 5 vertical movements from top to bottom.

This procedure was repeated three times.

2. TOPICAL ADMINISTRATION OF SODIUM ASCORBYL PHOSPHATE BY ACQUA-PEEL®

After abrasion, the samples of pig ear skin (surface area 2.54 cm^2) were mounted on diffusion cells (figure 1). The receiving compartments of the diffusion cells were filled with 10 ml of physiological solution (NaC1 0.9%). The surface of the abraded skin of the samples (n = 3 samples of skin per product) was treated with the anti-ageing product (MBAA009) and the bleaching product (MBE019) administered by means of microjets at 20 bar, 15 bar and 10 bar delivered by Acqua-Peel® for 45 seconds.

The anti-ageing (MBAA009) and bleaching (MBE013) products were administered (i) pure (3% SAP) or (ii) after dilution (1% SAP). In order to evaluate the method of administrating SAP by Acqua-Peel®, skin samples (n = 3 per product) were treated with 2 ml of each of the pure products (3% SAP) or diluted products (1% SAP) for 45 seconds in the **absence of microjets (control group).**

3. DETERMINATION OF CUTANEOUS PENETRATION

After treatment, the abraded skin samples were weighed, and then ground in 1.5 ml of a mixture of phosphate buffer and tetrahydrofuran, using a glass ball homogeniser (Minibearbeater®) for 60 seconds. The ground material was centrifuged for 5 minutes at 5000 rev/min. After filtration on a membrane (0.45 µm), the concentration of SAP in the filtrates was determined by high-performance liquid chromatography (HPLC) as described in report number R180603.

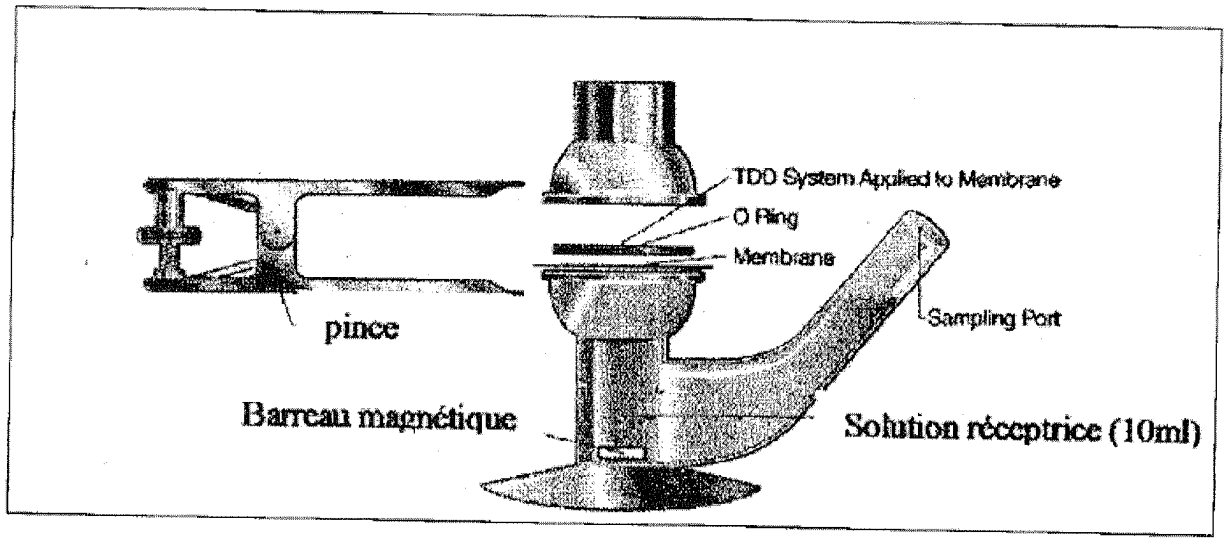

Figure 1: diffusion cell used in the present study as described in the technical protocol.

RESULTS

Figures 2-7 show the concentration of SAP (expressed in µg/g of fresh tissue) in the abraded skin after treatment with the anti-ageing product (MBAA009) and the bleaching product (MBE013) administered in the absence of or using microjets delivered by Acqua-Peel®. The pressure of the microjets was 20, 15 and 10 bar.

Pure anti-ageing product (MBAA009) (3% SAP)

Figures 2 and 4 show that the concentration of SAP in the skin treated with the pure (3% SAP) anti-ageing product (MBAA009) administered using microjets at 20 bar and 15 bar is significantly greater than that determined in the control group ($P<0.05$). However, the concentration of SAP after treatment with the pure (3% SAP) anti-ageing product (MBAA009) administered using microjets at 10 bar was not significantly different from that determined in the control group ($p>0.05$)(figure 6).

Pure (3% SAP) bleaching product (MBE013)

Figure 3 shows that the concentration of SAP in the skin treated with the pure (3% SAP) bleaching product (MBE013) administered using microjets at 20 bar is significantly greater than that determined in the control group ($P<0.05$). However, the

concentration of SAP after treatment with the pure (3% SAP) bleaching product (MBE013) administered using microjets at 15 and 10 bar was not significantly different from that determined in the control group (P>0.05) (figures 5 and 7)

Dilute (1% SAP) anti-ageing product (MBAA009) and bleaching product (MBE013)

Figures 2, 3 and 5 show that the concentration of SAP in the skin treated with anti-ageing (MBAA009) and bleaching (MBE013) products diluted with 1% SAP and then administered by means of microjets at 20 and 15 bar (experiment at 10 bar not carried out) was not significantly different from that determined in the control group (p>0.05)

Influence of the microjet pressure on the cutaneous absorption of SAP

Figures 8 and 9 show that the cutaneous concentration of SAP in the abraded skin treated for 45 seconds with the pure (3% SAP) anti-ageing (MBAA009) and bleaching (MBE013) products is correlated with the pressure of the microjets delivered by Acqua-Peel®. The cutaneous concentration of SAP in the abraded skin treated for 45 seconds with the pure (3% SAP) anti-ageing (MBAA009) and bleaching (MBE013) products administered at 20 bar is significantly greater than that determined by administration of the products at 15 and 10 bar. No significant difference was shown between the cutaneous concentrations of SAP after the administration of pure (3% SAP) products at 10 and 15 bar.

Captions to bar charts

Concentration of SAP (µg/g)

Test

Control

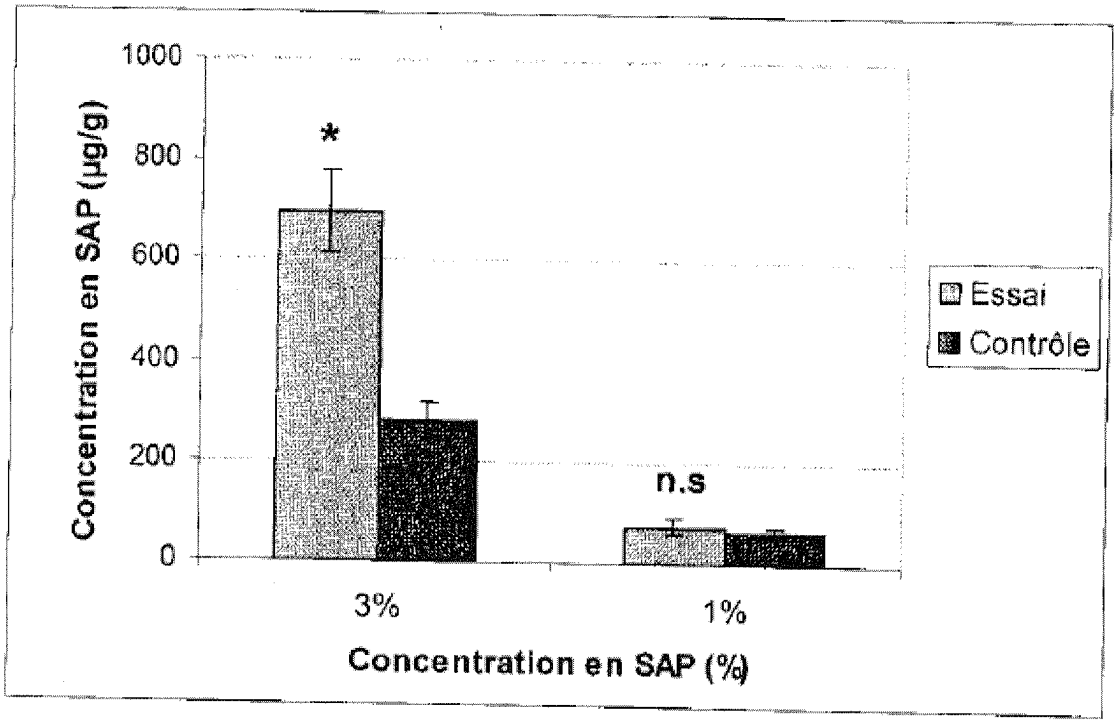

Figure 2: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) anti-ageing product (MBAA009) administered by means of microjets at 20 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

*: $p<0.05$ versus control group. n.s: not significantly different from control group.

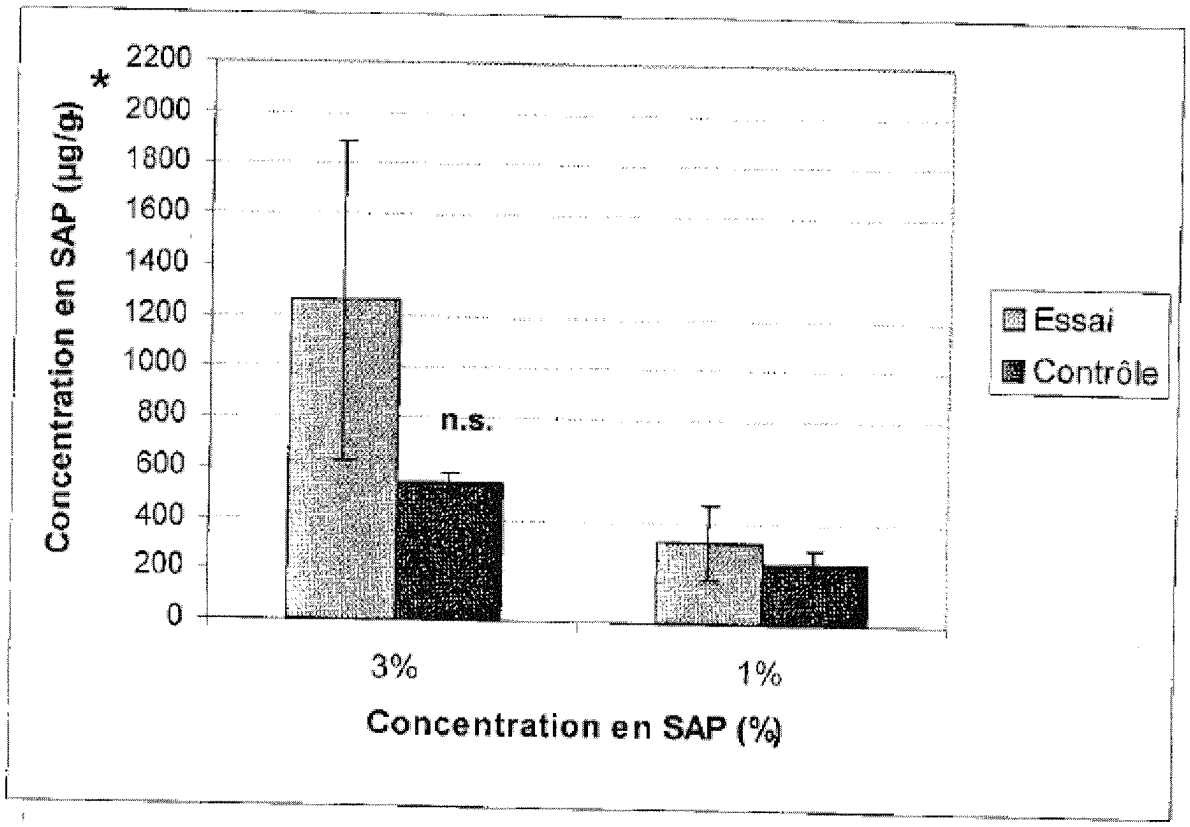

Figure 3: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) bleaching product (MBE013) administered by means of microjets at 20 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

*: p<0.05 versus control group. n.s: not significantly different from control group.

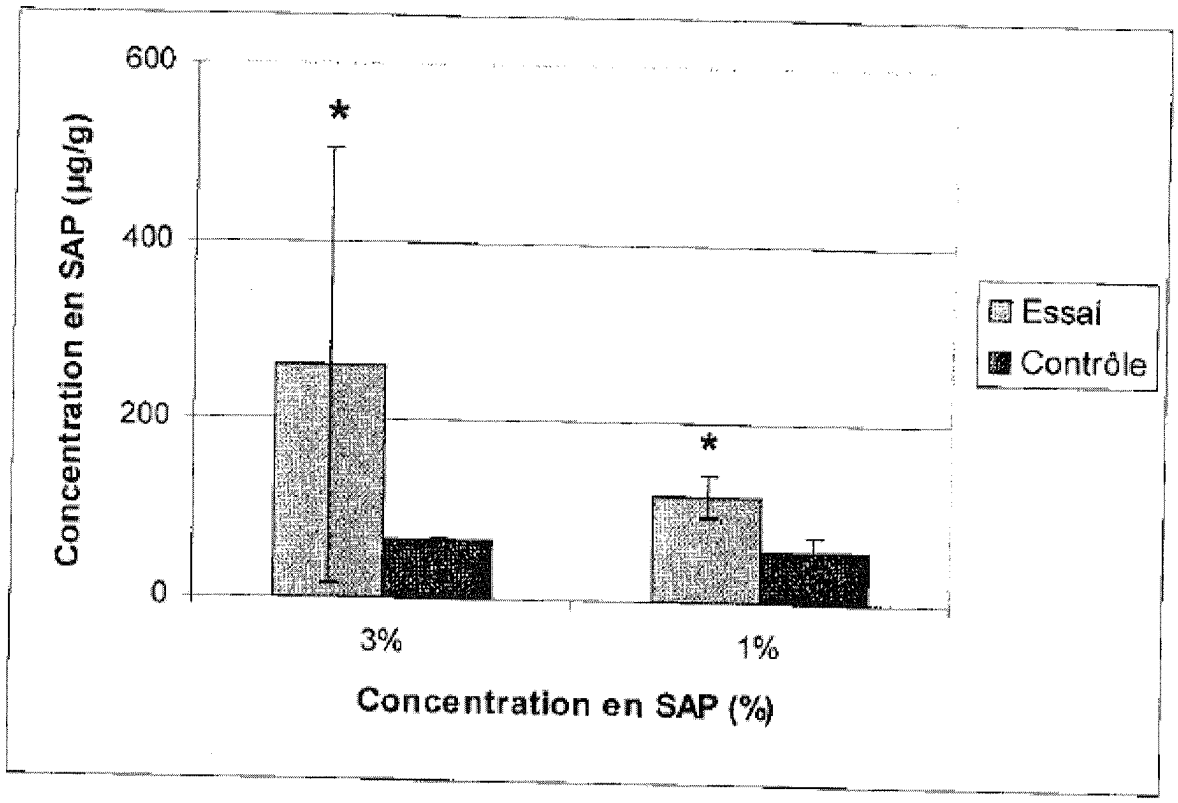

Figure 4: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) anti-ageing product (MBAA009) administered by means of microjets at 15 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

*: p<0.05 versus control group.

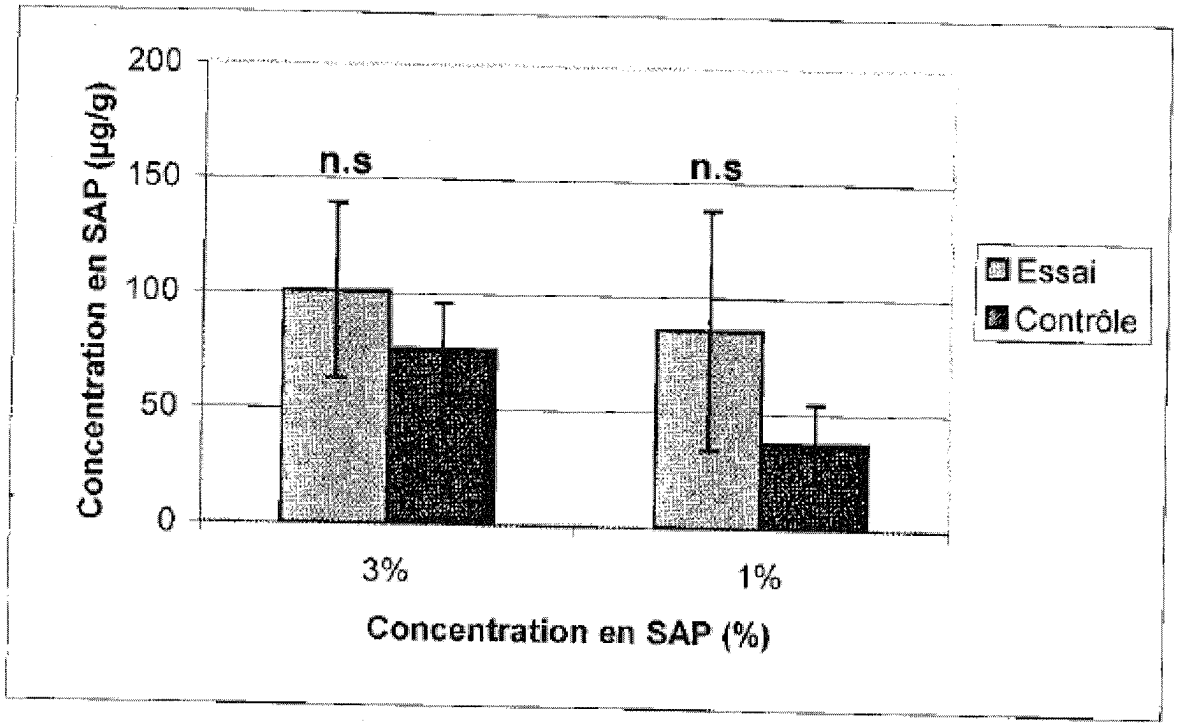

Figure 5: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) bleaching product (MBE013) administered by means of microjets at 15 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

n.s: not significantly different from control group.

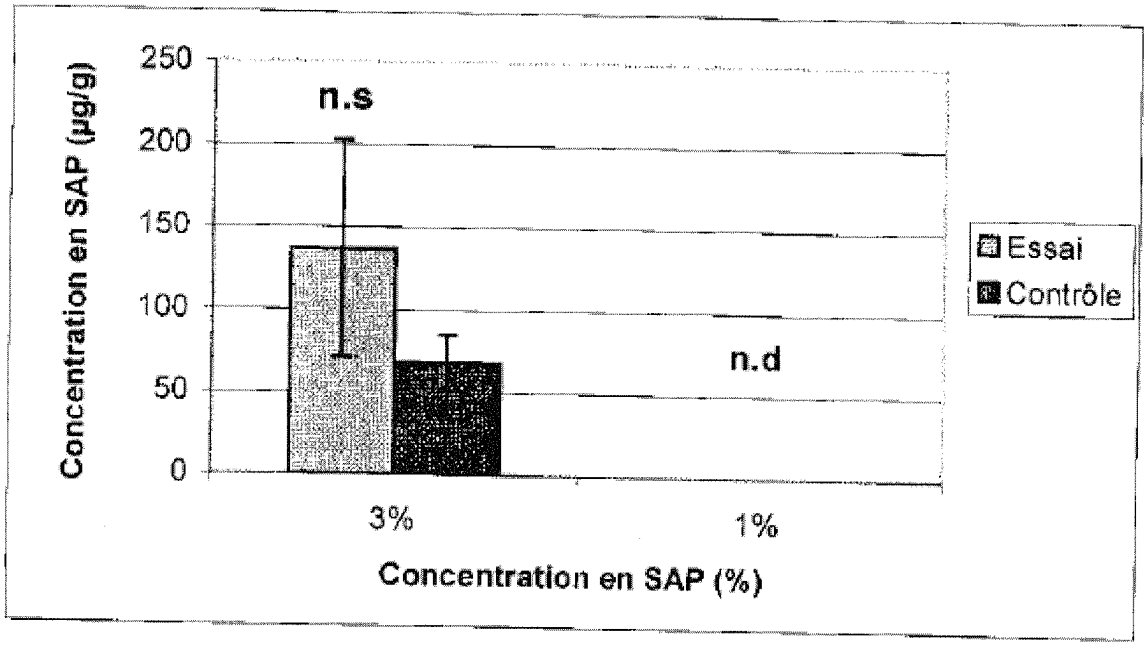

Figure 6: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) anti-ageing product (MBAA009) administered by means of microjets at 10 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

n.s: not significantly different from control group. n.d: not determined experimentally.

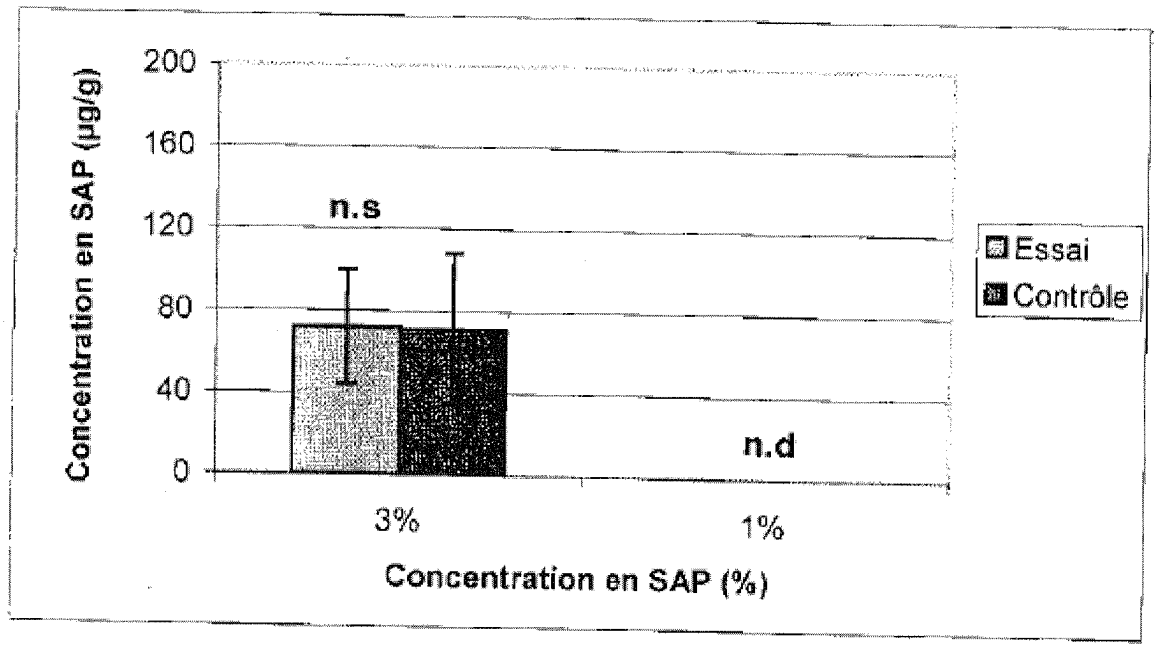

Figure 7: Cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with pure (3% SAP) or dilute (1% SAP) bleaching product (MBE013) administered by means of microjets at 10 bar (test) or in the absence of microjets (control).

Each data item is the mean ± standard deviation of three experimental determinations.

n.s: not significantly different from control group. n.d: not determined experimentally.

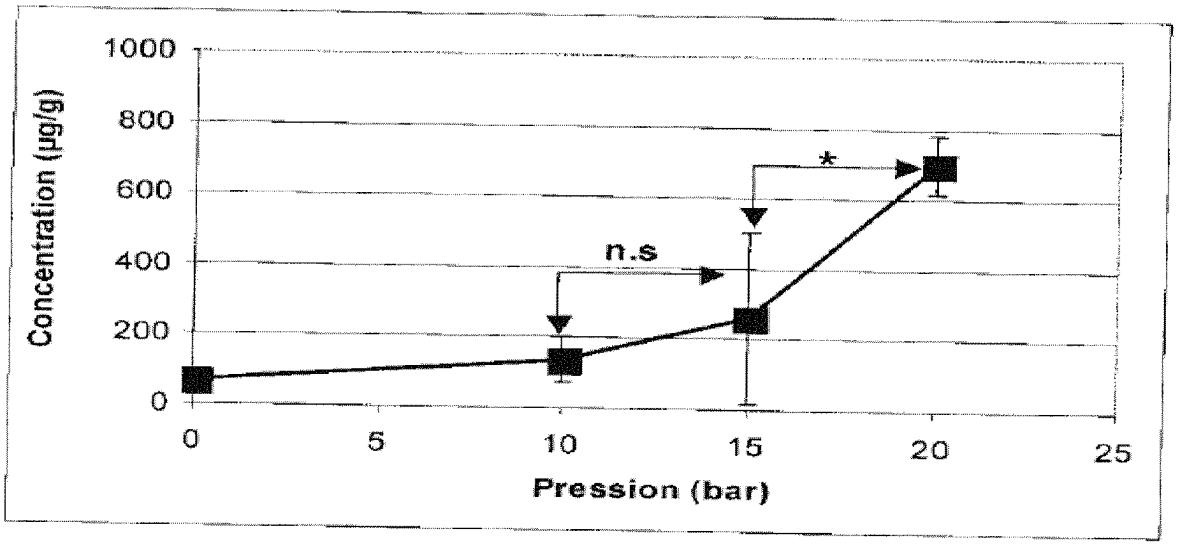

Figure 8: Correlation between the cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with a pure (3% SAP) anti-ageing product (MBAA009) as a function of the pressure of the microjets.

Each data item is the mean ± standard deviation of three experimental determinations.

*: $p<0.05$ versus 15 bar group. n.s: not significantly different from the 15 bar group.

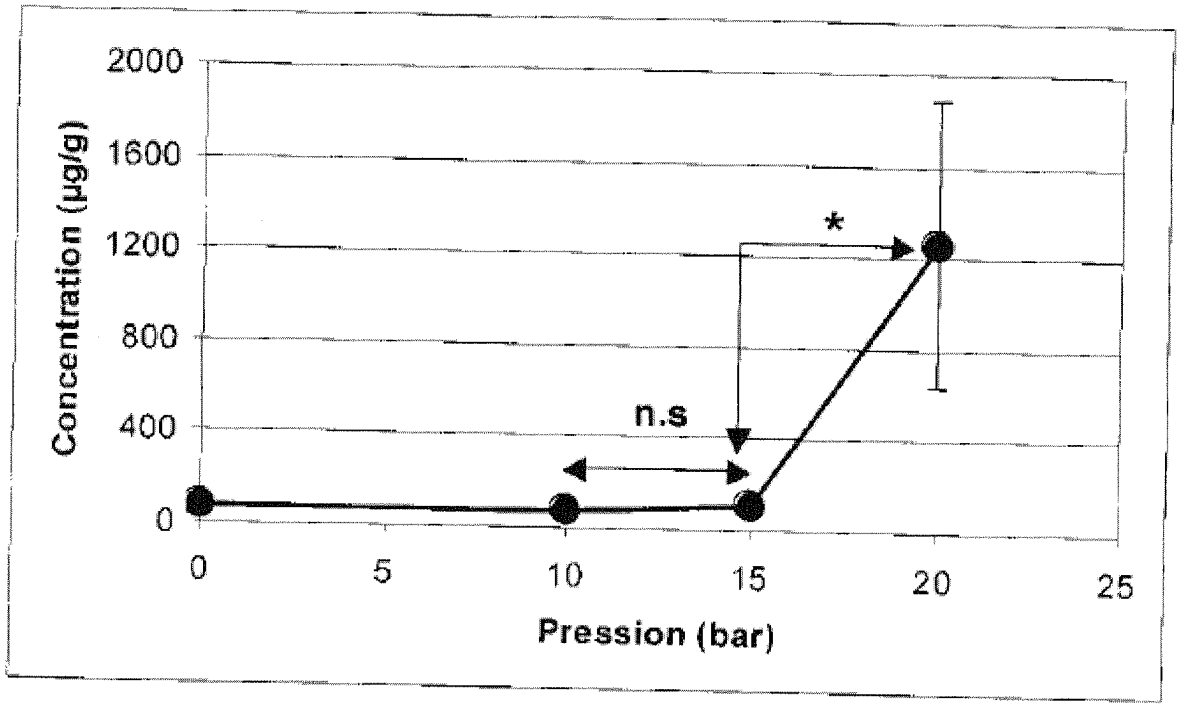

Figure 9: Correlation between the cutaneous concentration of SAP in the abraded skin (µg/g of fresh tissue) treated for 45 seconds with a pure (3% SAP) bleaching product (MBE013) according to the pressure of the microjets.

Each data item is the mean ± standard deviation of three experimental determinations.

*: $p<0.05$ versus 15 bar group. n.s: not significantly different from the 15 bar group.

HYDRODYNAMIC SODIUM ASCORBYL PHOSPHATE DELIVERY INTO THE SKIN

F. Pirot[1], V. Gaillard[1], V. Faivre[1], J-P. Devidal[2], F. Falson[1]

1 : Laboratoire de Recherche et Développement de Pharmacie Galénique Industrielle, EA 3741 *Ecosystèmes et Molécules Bioactives*, Faculté de Pharmacie, Université Claude Bernard Lyon I, 8 avenue Rockefeller, F-69373 Lyon cedex 08, France

2 : Medicamat, 59, avenue Augustin Dumont, 92240 Malakoff, France

1.Introduction

L-ascorbic acid (M.W. 176.1 g.mol^{-1}) has been used in skin care products for several decades, with claims of various benefits such as anti-aging, moisturizing, and skin whitening effects. Although *l*-ascorbic acid is a very hydrophilic compound with a partition coefficient between octanol and water of 0.020 ± 0.002, the skin permeation of *l*-ascorbic acid has been reported. However, the unstability of *l*-ascorbic acid implies the use a more stable derivative such as sodium ascorbyl phosphate (SAP). Recently, Water-Beam® was approved in European Market as a new device using epidermal abrasion and hydrodynamic drug delivery into the skin from water microjets.

In the present study, the usefulness of Water-Beam® for the topical hydrodynamic delivery of SAP was investigated *ex vivo* from different pressures of microjets.

2.Materials and Methods

Formulations

Two anti-aging and whitening cosmetic formulations containing 3% SAP were provided by IRFAQ (Courtaboeuf, France). Tested SAP concentration in formulations was 3% and 1% from appropriate dilution in distilled water.

Cutaneous absorption studies

Epidermis of porcine skin was abraded gently by using a powder of fruit pits projected delivered by Biopeel ® (Medicamat, Malakoff, France) at the skin surface following a standardized procedure [1] (Figure 1). Therefore, skin specimens were mounted in diffusion cell (Figure 2). The receptor compartment was filled with saline solution. An hand piece was introduced in the donor compartment (Figure 3) [2]. The hand piece was combined with a new patented device so-called Water-Beam® (Medicamat, Malakoff, France). SAP formulations were administered from microjets at 10-20 bar pressure onto the skin surface for 45 sec. An adjacent vacuum system allowed a re-circulation of SAP formulation during the skin treatment. Three or more skin specimens were used for each tested pressure. Three skin specimens were treated by 2 ml of SAP formulations as no-pressure experiment (control group).

After treatment, the skin specimens were homogenized in tube filled with a mixture of phosphate buffer (0.3 M, pH 4) and tetrahydrofuran (70:30, v/v) and glass beads. Therefore, the tubes were strongly agitated in Mini-Beadbeater® (Biospec Products, Bartlesville, USA). Finally, the tubes were centrifuged and the supernatants were filtered through 0.45 µm filters.

SAP content in supernatants was assessed by high performance liquid chromatography (HPLC). The mobile phase was a mixture of phosphate buffer (0.3 M, pH 4) and acetonitrile (60:40, v/v). SAP assays were carried out by reversed-phase adsorption chromatography using a Lichrosorb NH_2® (Li-NH_2, 7 µm, 250 x 4 mm, Interchim, Montluçon, France). The sample volume injected was 20 µl. The mobile phase flux was 0.8 ml/min. Detection was performed at 258 nm. The retention time of SAP was 5.3 min.

Statistical analysis of in vivo study

SAP content in skin specimens treated from hydrodynamic delivery was compared to that reported in control group by Student's test. The chosen level of significance was $p < 0.05$.

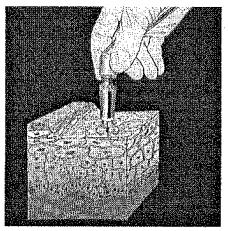

Figure 1: Schematic representation of epidermal abrasion using Biopeel®.

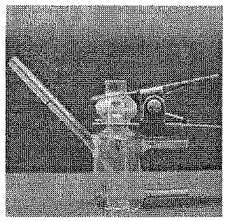

Figure 2: Diffusion cell used in cutaneous absorption studies of SAP in ear porcine skin.

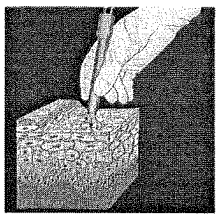

Figure 3: Schematic representation of hydrodynamic delivery of SAP formulation by using Water-Beam®.

3.Results and Discussion

Cutaneous absorption studies

As shown in Table 1, the hydrodynamic delivery increased the penetration of SAP into abraded skin as a function of micro-jet pressure. A significant increase of SAP content in skin specimens treated by 1% and 3% SAP in anti-aging formulation delivered at 15 and 20-bar, respectively was shown as compared to control group.

Table 1: Comparison of SAP content in skin treated SAP formulations delivered by Water-Beam® or by passive diffusion. Each data is the mean ± standard deviation of 3 or 4 experimental determinations. Control data are reported between brackets.
* : $p < 0.05$ versus control group; ** : $p < 0.01$ versus control group (Student's test).

Formulations	Hydrodynamic pressure (bar)		
	10	15	20
Anti-aging			
3%	137 ± 66	261 ± 244	698 ± 82**
	(68 ± 17)	(67 ± 1)	(282 ± 36)
1%	nd	119 ± 24*	74 ± 17
	nd	(58 ± 17)	(64 ± 10)
Whitening			
3%	72 ± 28	101 ± 38	1258 ± 629
	(71 ± 37)	(76 ± 20)	(541 ± 38)
1%	nd	86 ± 52	320 ± 146
	nd	(37 ± 17)	(240 ± 51)

nd: not determined

The hydrodynamic SAP delivery reinforced the drug disposition in abraded skin as a function of micro-jet pressure in the range of donor concentration (3% versus 1%).

4.Conclusion

The present study showed that the hydrodynamic administration might constitute a promising approach for the improvement of cutaneous drug delivery of highly hydrophilic drug.

5.References

1. Divaris M. Dermal improvement via aquapressure: DIVA protocol. Int J Cosmet Surg, 2003, 5(2): 185-191.
2. Pirot F., Martinez Comendador S., Benvenuto M., Basset V., Gaillard V., Faivre V., Devidal J-P., Falson F. Rapid local anesthesia by hydrodynamic delivery of lidocaine. *Stratum Corneum IV* Congress, June 17-19th, 2004, Paris.

CLAUDE BERNARD UNIVERSITY - LYON I

Faculty of Pharmacy
Institute of Pharmaceutical and Biological Sciences

8, avenue Rockefeller - 69373 LYON Cedex 08 - Tel. 04 78 77 71 67 - Fax 04 78 77 72 47

PHARMACEUTICAL TECHNOLOGY, BIOPHARMACY AND COSMETOLOGY LABORATORY

F. FALSON, Professor
F. PIROT, Associate Professor, Attaché to the Civil Hospices in Lyon

REPORT: *EX VIVO* STUDY OF THE FUNCTIONAL CONDITION OF THE SKIN FOLLOWING ADMINISTRATION OF DEMINERALISED WATER

Biological Samples

The biological samples necessary for carrying out the *ex vivo* trials came from pig ear samples taken at the Physiology Laboratory of Dr Q. TIMOUR (CLAUDE BERNARD UNIVERSITY - LYON I). The pig ears were retrieved by the Principal Investigator with the agreement of the Head of the Physiology Laboratory, 16 hours before the trial. The ears were kept at +4°C until the start of the trial. Ears which were not used in the study were preserved at -18°C.

Product Studied:

- Demineralised water

TECHNICAL PROTOCOL

1.1 *Ex vivo* study of skin hydration after treatment with demineralised water administered by Acqua-Peel®

The aim of this study was to show whether the topical administration of demineralised water using microjets delivered by Acqua-Peel® significantly modifies the level of skin hydration. An area of skin was treated for 1.5 min, 2 min and 5 min with demineralised water administered topically using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar).

After treatment, the skin hydration was determined using a Corneometer CM 825. The skin hydration values were expressed in arbitrary units (AU). The skin hydration obtained using microjets was compared with that obtained in the absence of microjets.

1.2 *Ex vivo* study of the transepidermal water loss (TEWL) of the skin after treatment with demineralised water administered by Acqua-Peel®

The aim of this study was to show whether the topical administration of demineralised water using microjets delivered by Acqua-Peel® significantly modified the transepidermal water loss (TEWL).
An area of skin was treated for 1.5 min, 2 min and 5 min with demineralised water administered topically using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar).

After treatment, the TEWL of the skin was determined using a Tewameter. The TEWL obtained using microjets will be compared with that obtained in the absence of microjets.

RESULTS AND DISCUSSION

1.1 *Ex vivo* study of skin hydration after treatment with demineralised water administered by Acqua-Peel®

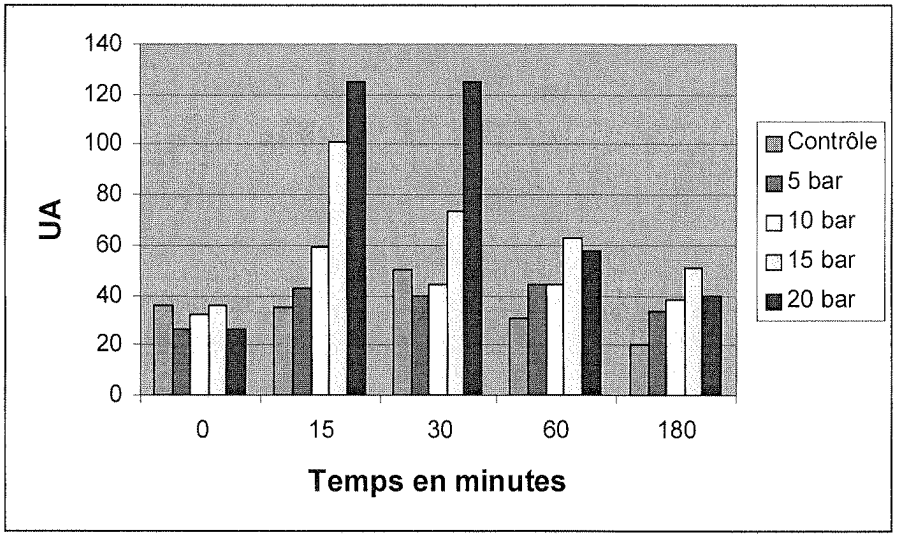

Figure 1: *Ex vivo* study of skin hydration (expressed in arbitrary units, AU) as a function of time after treatment with demineralised water administered for 1.5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.

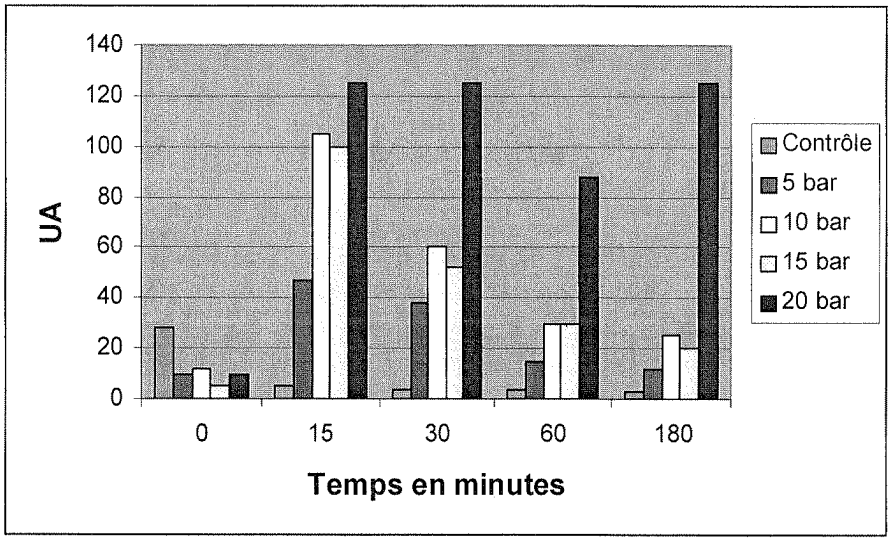

Figure 2: *Ex vivo* study of skin hydration (expressed in arbitrary units, AU) as a function of time after treatment with demineralised water administered for 2 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.

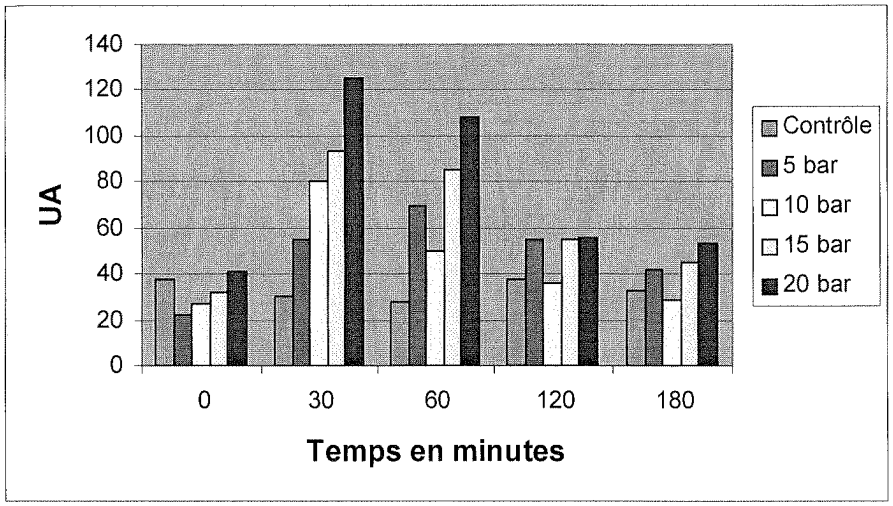

Figure 3: ***Ex vivo*** **study of skin hydration (expressed in arbitrary units, AU) as a function of time after treatment with demineralised water administered for 5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.**

Figures 1-3 show the skin hydration values as a function of time after treatment with demineralised water administered for 1.5 min, 2 min and 5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). The administration of demineralised water by Acqua-Peel® for 1.5 min, 2 min and 5 min increases skin hydration for almost 3 h compared to the control group (demineralised water in the absence of pressure). This increase in skin hydration depends on the pressure of the microjets.

1.2 *Ex vivo* study of the transepidermal water loss (TEWL) of the skin treated with demineralised water administered by Acqua-Peel®

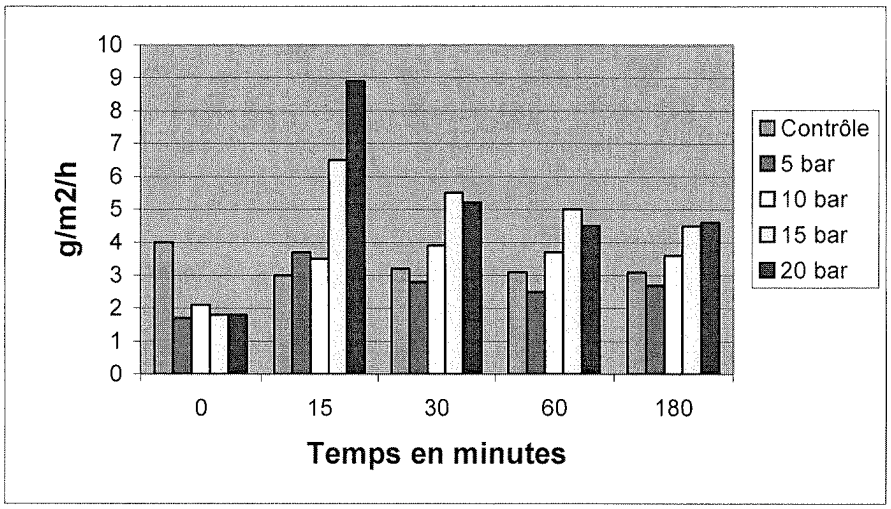

Figure 4: ***Ex vivo*** **study of the TEWL (expressed in g/m^2/h) as a function of time after treatment with demineralised water administered for 1.5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.**

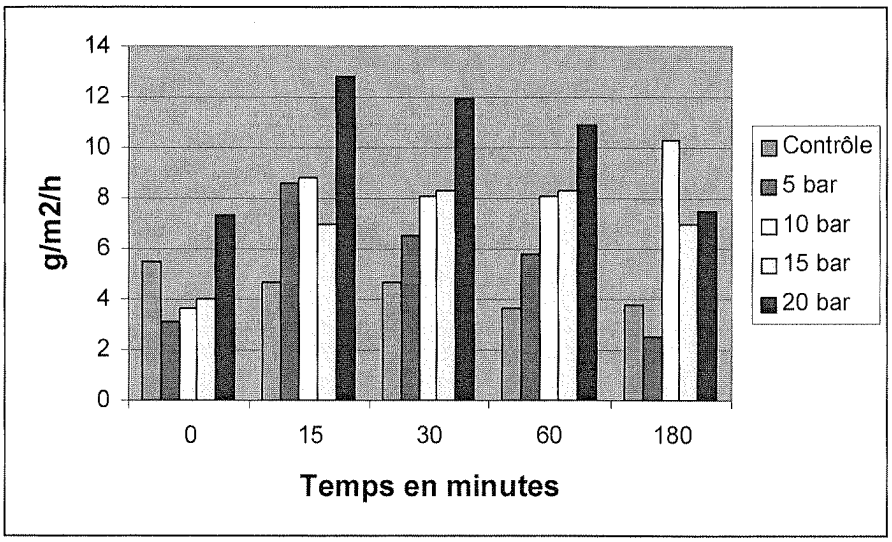

Figure 5: *Ex vivo* study of the TEWL (expressed in g/m²/h) as a function of time after treatment with demineralised water administered for 2 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.

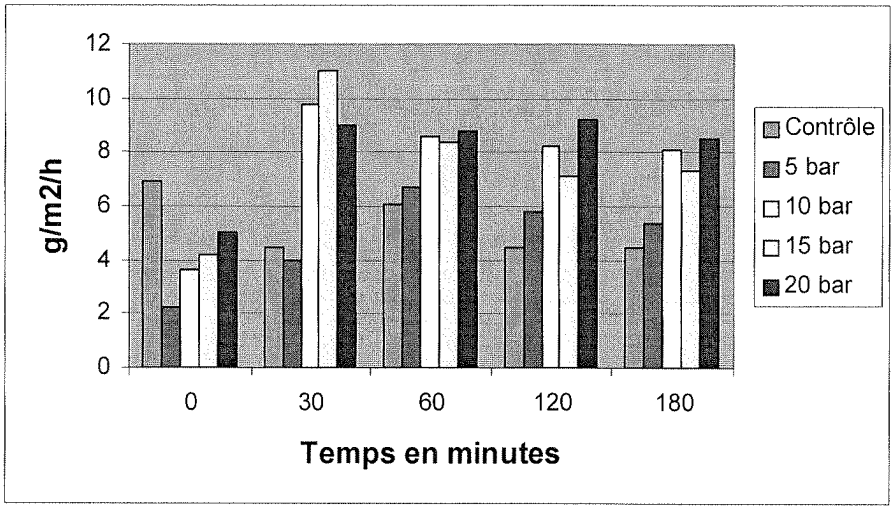

Figure 6: *Ex vivo* study of the TEWL (expressed in g/m²/h) as a function of time after treatment with demineralised water administered for 5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). Each value is the average of 3 experimental determinations.

Figures 4-6 show the transepidermal water loss (TEWL) values as a function of time after treatment with demineralised water administered for 1.5 min, 2 min and 5 min using microjets delivered by Acqua-Peel® (pressure 0, 5, 10, 15 and 20 bar). The administration of demineralised water by Acqua-Peel® for 1.5 min, 2 min and 5 min increases the TEWL for almost 3 h compared to the control group (demineralised water in the absence of pressure). This increase in TEWL depends on the pressure of the microjets.
At low pressure (5 bar), the increase in TEWL is transient, regardless of the skin exposure time (1.5 min, 2 min and 5 min). After 3 h, these TEWL values are highly comparable to those obtained in the control group.

Conclusion
At the end of this study and under the experimental conditions described above, it is clear that the administration of demineralised water at low pressure (5 bar) for 5 min does not appear to modify the

functional condition of the skin. By contrast, an administration of demineralised water at a higher pressure (> 5 bar) appears to modify the functional properties of the skin and in particular the skin barrier function which is evaluated by the considerable variations in TEWL compared to the control group.

Legend for figures:

FRENCH	ENGLISH
Temps en minutes	Time in minutes
UA	AU
Contrôle	Control